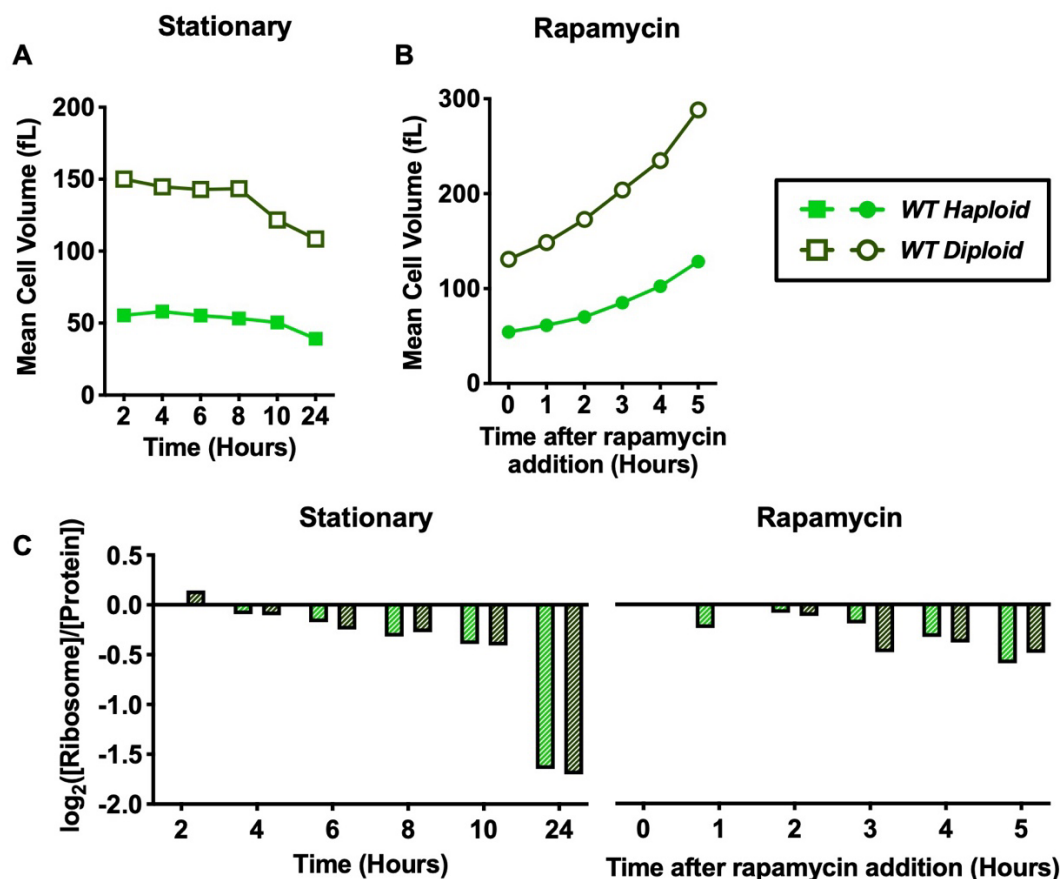


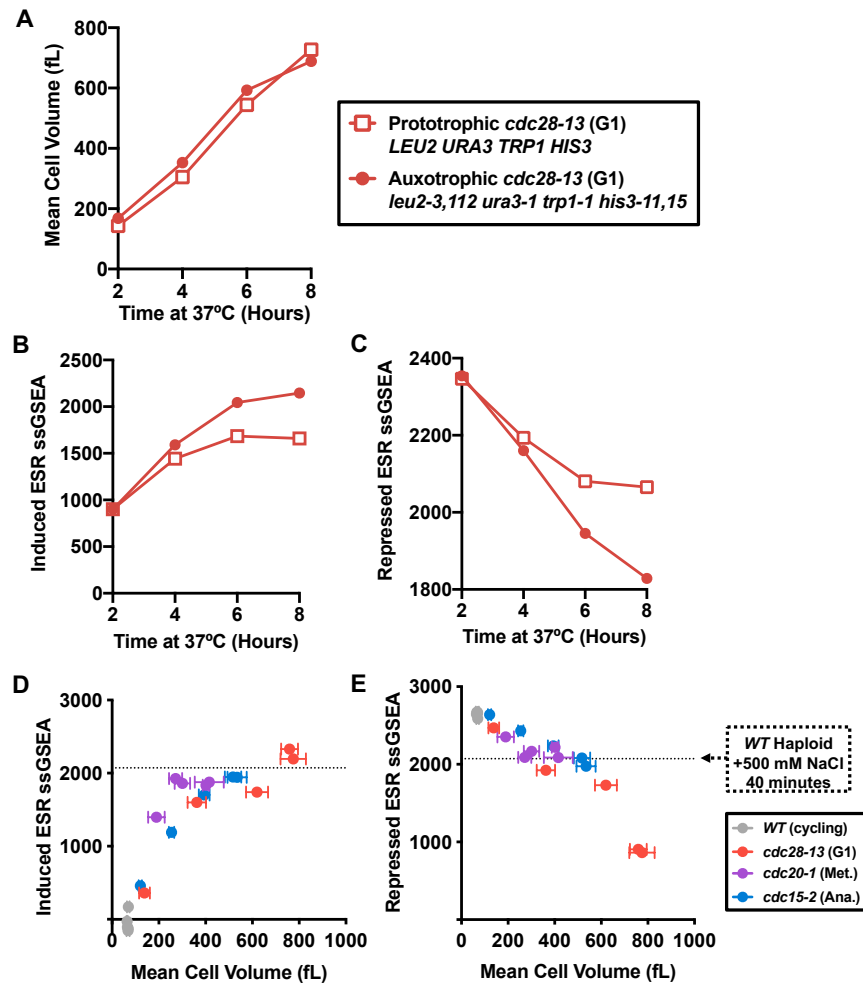
**Figure S1. Macromolecular crowding in *cdc-ts* mutants arrested in the cell cycle.**

*WT* (gray, A2587), *cdc28-13* (red, A39000), *cdc20-1* (purple, A937), and *cdc15-2* (blue, A2596) cells were grown to log phase in YEPD at 25°C and then shifted to 37°C for 6 hours. *WT* cultures were kept in log phase, termed cycling, at  $OD_{600nm} = 0.2-0.8$ , by diluting with pre-warmed (37°C) YEPD. GEM diffusion was performed as in (Delarue et al., 2018) to calculate median diffusion coefficients. Error bars represent range of two experimental replicates.



**Figure S2. Comparison of protein and ribosome quantification in haploid and diploid *WT* cells.**

For stationary phase experiments, *WT* haploid (green, A2587) and *WT* diploid (dark green, A33728) cells were grown in YEPD for 24 hours at 30°C. For rapamycin experiments, *WT* haploid (green, A2587) and *WT* diploid (dark green, A33728) cells were grown to log phase in YEPD at 30°C. 5 nM rapamycin was added to *WT* haploid cells, and 2.5 nM rapamycin was added to *WT* diploid cells. Cells were grown for 5 hours at 30°C in the presence of rapamycin. **(A-B)** Mean cell volume (fL) was measured for *WT* haploid (green) and *WT* diploid (dark green) cells grown **(A)** into stationary phase (squares) and **(B)** in the presence of rapamycin (circles). **(C)** Protein and ribosome concentrations were quantified using the method described in (Terhorst et al. , 2017). [Ribosome]/[Protein] was determined. Values were normalized to the 2-hour time point in stationary experiments and to the 0-hour time point in rapamycin experiments and subsequently  $\log_2$  transformed.



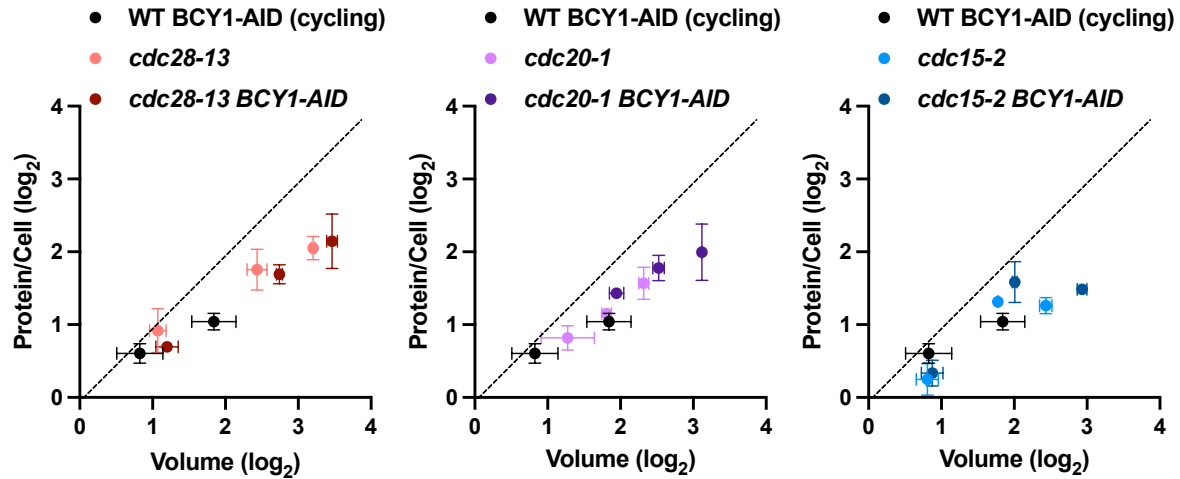
**Figure S3. Comparison of ESR activation in prototrophic *cdc28-13* cells and auxotrophic *cdc28-13* cells.**

Prototrophic *cdc28-13* (red squares, A41270) and auxotrophic *cdc28-13* (red circles, A17896) were grown to log phase in YEPD at 25°C. Cells were synchronized with alpha factor then shifted to 37°C for 6 hours.

(A) Mean cell volume (fL) was measured for prototrophic *cdc28-13* cells (red squares) and auxotrophic *cdc28-13* cells (red circles).

(B-C) RNA-Seq samples were collected, and gene expression data were analyzed by calculating ssGSEA projection values for the (B) induced ESR and (C) repressed ESR.

(D-E) Induced and repressed ESR ssGSEA values from Figure 3A and 3B plotted against mean cell volume (mean  $\pm$  SD) shown in Figure 1A. The induced ESR increases linearly with volume until reaching an apparent plateau that coincides with the positive control. No such plateau effect is apparent in the repressed ESR.



**Figure S4. ESR suppression does not prevent cytoplasm dilution.**

Protein amount per cell normalized to WT cycling cells as shown in Figure 5F plotted against cell volume shown in Figure 5A-C. The dotted line indicates perfect scaling of protein content with cell volume. WT-BCY1-AID data is identical on all graphs. Error bars represent the range of two experimental replicates.

Name	Number	Genotype	Source
<i>WT</i> Haploid	A2587	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+</i>	Nasmyth Lab
<i>cdc28-13</i>	A39000	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, cdc28-13::URA3</i>	Amon Lab
<u><i>cdc20-1</i></u>	A937	<i>MATalpha, cdc20-1, ura3, trp1, leu2, his3, ade2, can1</i>	Nasmyth Lab
<i>cdc15-2</i>	A2596	<i>MATa, cdc15-2, leu2-3, ura3, trp1-1, omns, ade1</i>	Nasmyth Lab
<i>WT GEM</i>	LH4415	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+ HIS3::pINO4-PfV-Sapphire</i>	Holt Lab
<i>cdc28-13 GEM</i>	LH4416	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, cdc28-13::URA3 HIS3::pINO4-PfV-Sapphire</i>	Holt Lab
<i>cdc20-1 GEM</i>	LH4417	<i>MATalpha, cdc20-1, ura3, trp1, leu2, his3, ade2, can1 HIS3::pINO4-PfV-Sapphire</i>	Holt Lab
<i>cdc15-2 GEM</i>	LH4418	<i>MATa, cdc15-2, leu2-3, ura3, trp1-1, omns, ade1 HIS3::pINO4-PfV-Sapphire</i>	Holt Lab
<i>bar1Δ</i>	A2589	<i>MATa, bar1::HisG, leu2-3, ura3, trp1-1, his3, ade2, can1-100, GAL, psi+,</i>	Nasmyth Lab
<i>WT</i> Diploid	A33728	<i>MATa/alpha, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+</i>	Fink Lab
<i>cdc28-13</i> Auxotroph	A17896	<i>MATa, cdc28-13, ADE2, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+</i>	Amon Lab
<i>cdc28-13</i> Prototroph	A41270	<i>MATa, cdc28-13, ADE2, URA3, TRP1, HIS3, can1-100, GAL, psi+, cdc28-13, leu2::4xSTRE-GFP:LEU2</i>	Amon Lab
<i>WT</i> <i>AID-BCY1</i>	A40439	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, KanMX:pRFA1:9Myc-AID-BCY1, leu2::pTEF1-osTIR::LEU2</i>	Amon Lab
<i>cdc28-13</i> <i>AID-BCY1</i>	A40444	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, cdc28-13::URA, KanMX:pRFA1:9Myc-AID-BCY1, leu2::pTEF1-osTIR::LEU2</i>	Amon Lab
<i>cdc20-1</i> <i>AID-BCY1</i>	A40499	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, cdc20-1, KanMX:pRFA1:9Myc-AID-BCY1, leu2::pTEF1-osTIR::LEU2</i>	Amon Lab
<i>cdc15-2</i> <i>AID-BCY1</i>	A40501	<i>MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+, cdc15-2, KanMX:pRFA1:9Myc-AID-BCY1, leu2::pTEF1-osTIR::LEU2</i>	Amon Lab

**Table S3. Yeast strains used in this study.** Description of the strain names, numbers, genotypes, and source used in this paper.